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TITLE: METHOD AND APPARATUS FOR HOLOGRAPHIC REFRACTOMETRY

5       AREA OF INVENTION

The present invention relates to a method and device
for holographic refractometry useful for the comparision of
refractive index between an object and a surrounding medium
using digital holography.

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PRIOR ART

A commonly known method for determination of refrac-
tive index of small inhomogenous, transparent objects is
the method known as the Becke method. The Becke method in-
15    volves placing the object in a medium having a known re-
fractive index, for example a Refractive Index Liquid. The
sample is arranged in a microscope and light is passed
through the sample. The person using the microscope moves
the focal plane upwards (or downwards). The Becke lines
20    seem to move outwards or inwards relative to the contour of
the object. The movement of the Becke lines is dependent on
whether the refractive index of the object is lower or
higher than the refractive index of the surrounding medium.
By placing the object in several media having different re-
25    fractive index and observing when the movement of the con-
tour change direction as the focal plane is moved upwards,
the refractive index of the object may be determined. Thus,
the Becke method of determining refractive index of an ob-
ject is an indirect method.

30       The Becke method involves that a person has to move
the focal plane of the microscope by hand while observing
the movement of the contours of the object. Thus, the
method does not lend itself for automation. Moreover, the
analysis has to be performed at site. Thus, it is not
35    possible to make an image of the object and to analyze the
image afterwards in another environment or at another time.

Other methods of measuring refractive index are the
phase contrast method and the oblique illumination method,
see for example W. D. Nesse, "Introduction to optical
Mineralogy", Oxford University Press, New York, second edi-
5   tion, 1991.

Refractometry has applications in different sciences.
The index of refraction is material specific, which is use-
ful in identification of different substances in a blend.
In pharmacology, particle size characterization and distri-
10  bution of chemical substances play an important role in re-
search and manufacturing. In mineralogy, the index of re-
fraction is an important parameter in classifying different
minerals.

Holographic methods are common today. For example
15  DE2133803 discloses a holographic method according to which
refractive index changes can be observed. The document does
not deal in detail with how such measurement is performed.


SUMMARY OF THE INVENTION
20      Accordingly, it is an objective of the invention to
provide a method and a device that makes it possible to
automate the process of measuring the refractive index of
an object.

Another objective of the invention is to provide a
25  method and device that enables remote analyzing of the re-
fractive index of an object at any time after that an image
of the object has been obtained.

These objectives are met with a method and device
for determining refractive index of an object compared to a
30  refractive index of a surrounding medium, by exposing said
sample to a laser object beam and letting the object beam
interfere with a laser reference beam to obtain a hologram,
analyzing the hologram for phase information, and determin-
ing if the refractive index of the object is higher or
35  lower than the refractive index of the surrounding medium

based on said phase information. The method may be per-
formed by a computer.

   The method may be used for counting the number of
particles having a first refractive index and counting the
5   number of particles having a second refractive index in a
specific area of said sample if the object has particles of
a first substance having a first refractive index and a
second substance having a second refractive index and a me-
dium having a refractive index between said first and
10  second refractive index. In this way the relationship be-
tween the substances may be calculated.


   BRIEF DESCRIPTION OF THE DRAWINGS
   Further objects, features and advantages of the in-
15  vention will become apparent from the following description
of several embodiments of the invention with reference to
the drawings, in which:
   Fig. 1 is a schematic diagram of an experimental
setup for performing the method according to the invention,
20  Fig. 2 is a schematic diagram of an object comprising
wave fronts moving beyond the object,
   Fig. 3 is a schematic diagram showing a phase analy-
sis of a hologram obtained of the object of Fig. 2,
   Fig. 4 is a photograph showing a microscope view of a
25  sample comprising objects according to the invention, and
   Fig. 5 is a photograph showing a microscope view of a
sample illustrating an aspect of the invention.


   DETAILED DESCRIPTION OF EMBODIMENTS
30  Fig. 1 is a schematic diagram of an experimental
setup of a Fourier holography equipment. The equipment com-
prises a JDS Uniphase 10 mW He-Ne laser 17 emitting light
at the wavelength of 633 nm. The light passes a shutter 6,
a first polarizer 5 and a second polarizer 4. By the polar-
35  izers, the intensity of the laser beam may be adjusted.

Thence, the laser beam passes a first halfwave plate 3 and
reaches a polarizing beam splitter 2 dividing the beam into
an object beam 14 and a reference beam 15. The object beam
14 passes a second halfwave plate 7 and is diverted 90 de-
5    grees by a mirror 10. Thence, the object beam passes an
Iris diaphragm 16 and reaches a beam splitter 9. The refer-
ence beam 15 is diverted by a mirror 1 towards the beam
splitter 9 and passes a GRIN lens 11, which diverts the
reference beam 15 as shown in the enlarged section of Fig.
10   1.

An object 12 is arranged close to the beam splitter
9. The laser beam passing through the object is collected
by a sensor 8. The reference beam is diverted towards the
sensor 8 by the beam splitter 9, whereby the sensor 8 com-
15   prehends the reference beam as originating from a virtual
point source 13 close to the object.

By means of the two halfwave plates, the intensity in
the reference beam and the object beam may be controlled
and at the same time keep the polarization of the fields
20   linear.

The experimental digital holograpy setup implies a
spherical reference point source close to the illuminated
object. This gives approximately the same curvature of the
reference field and the object field and hence low spatial
25   frequencies of the hologram. To achieve a large numerical
aperture of the reference field and keep the point source
close to the object, a 0.25 pitch GRIN lens is used to
widen the reference beam. The GRIN lens has a numerical
aperture of 0.37, a diameter of 1.0 mm and is placed in a
30   holder close to the beam splitter. The reference point
source is thereby reflected by the beam splitter to the
sensor. The arrangement with the beam splitter gives a vir-
tual point source which can be placed close to the object.

In the experimental setup, it is not possible to
35   measure the object field directly. Instead an approximation

of the object field and the reference field, i.e. a sampled
version of the interference pattern, is measured by the
sensor. The object field is used to determine an image of
the object. The evaluation of the object field is performed
in a computer by means of FFT (fast Fourier transforms).

The experimental setup described so far may be
altered in different applications. For example, the Iris
diafragm 16 may be omitted as well as the members 3, 4, 5,
6 and/or 7. The laser beams can be confined to a closed re-
gion with optical fibers and hence eliminate the mirrors 1
and 10, etc.

In the method according to the invention, it is
possible to see transparent objects, such as biological
specimens, without any preparation. To the contrary, in the
prior art, specimens are routinely stained. This approach
is unsatisfactory in many respects. The stain, for example,
kills a specimen whose life processes may be under study.

In Fig. 2, a transparent object is shown. The object
does not absorb any light, but the light that travels
through the object will experience a difference in the op-
tical path length. The wave front that emerges from the ob-
ject will thus be phase shifted. This distortion can be de-
tected with holography as phase differences and for example
be represented as a hue difference on a computer printout.

Fig. 3 shows a hologram print-out of the object
according to Fig. 2, in which the different heights are
displayed as different phases represented by different
colors. Thus, the inner circle may be blue 41, the next
ring 42 green, the following ring 43 yellow and the outer
ring 44 red.

Fig. 4 is a phase image of lactose crystals mounted
in a liquid with $n_m$ = 1.542 at the wavelength 589 nm. The
phase increases (decreases) when going from an object with
higher (lower) refractive index to an object with lower
(higher) refractive index. The photograph at the top is the

unwrapped phase image and the photograph at the bottom is the re-wrapped phase image.

In the present invention, a single hologram is obtained. A hologram comprises information of several focal planes. Consequently, it is possible to maintain the hologram and later analyze it according to the Becke method. The computer may be programmed to produce images of the crystals at different focal depth and the movement of the contours of the crystals may be visualized.

Furthermore, it is observed that a hologram comprises phase information. By programming the computer to indicate the phase of the laser light from the object, it is possible to generate an image, in which the phase is displayed, for example as different colors or hues.

It has been observed that the phase order is different depending on whether the object has a refractive index, which is larger or smaller than the surrounding liquid refractive index.

Thus, the index of refraction of an isotropic object can be determined with repeated measurement where the surrounding medium or liquid is changed until the phase order has been reversed. The index of refraction for the object is then between the indexes of refraction for the two liquids having different phase order.

Moreover, two different isotropic objects can be separated if the surrounding liquid is matched somewhere between the indexes of refraction for the two objects.

Such separation may for example be performed for chrystals of Sodium Chloride NaCl and chrystals of Potassium Chloride KCl. Chrystals having an average size of about 40 $\mu$m were used. These chrystals are isotropic, i.e. they have one index of refraction irrespectively of the direction of illumination. Their respective indices of refraction are denoted $n_{NaCl}$ and $n_{KCl}$. The crystals are mounted in a refractive index liquid with a refractive index de-

noted $n_m$, such that the refractive indices are ordered as $n_{KCl} < n_m < n_{NaCl}$.

The phase is changed when going through the crystals. The phase shift can be observed as a color change from

5   black, over gray, to white and back to black.

To obtain an image that depicts the optical thickness of the sample, the phase is unwrapped and the large scale phase variation of the illuminating wave is removed by fitting the unwrapped phase image to a polynomial. In these

10   unwrapped and background corrected phase images the images depict the contrast in refractive index times the propagation length through each crystal. Moreover, to improve the interpretation of the unwrapped background corrected phase images, the phase is plotted as a contour plot with $2\pi$ be-

15   tween each contour. In the contour plot, positive values are represented as darker colors and negative values as brighter colors; see Fig. 5.

In this way, the crystals may be counted or the volume distribution may be calculated.

20   Anisotropic objects have different indexes of refraction in different directions. The co-polarized part of the scattered light from an anisotropic object seems to have been scattered by an isotropic object with an effective index of refraction n´. By multiple exposures of the same ob-

25   ject with a step by step rotation of the polarization of the illuminating light, the smallest and the largest of the principal refractive indexes can be extracted. The anisotropic objects considered here are uniaxial, i.e. they have one optical axis where the index of refraction varies with

30   polarization.

Fig. 4 shows an object consisting of lactose crystals. These crystals are anisotropic and have different retraction indexes in different directions. These crystals are arranged in a liquid having a refractive index of

35   1.542, for example a Cargille Refractive Index Liquid that

may be obtained with predefined refractive index. The
wrapped phase image is improved by an approximation to a
two-dimensional phase unwrapping algorithm, whereby large
scale phase variation of the illuminated wave is removed.
5   Finally, the unwrapped phase image may be re-wrapped to the
range of $-2\pi$ to $+2\pi$.

The image is calculated by a computer and may be dis-
played as different shades of gray or as colors. The com-
puter may be programmed to determine the number of objects
10  that have a refractive index larger than or smaller than
the refractive index of the surrounding medium.

As appears from Fig. 4, some of the particles are
white and some of the particles are black, indicating
whether the refractive index of the relevant particle is
15  above or below the refractive index of the  surrounding me-
dium.

For example, if the method is to be used for deter-
mining the relationship between the number of particles of
two isotropic substances having different refractive index,
20  a medium is used with a refractive index in between the re-
fractive indexes of the substances. In the processed holo-
gram, the substances will appear as particles having dif-
ferent gray shade compared to the surrounding medium, for
example substantially white and substantially black par-
25  ticles. The computer may be programmed to count the number
of particles of each type on a specific surface area of the
sample. Thus, the relationship between the two substances
may be fully automatically calculated by the computer. The
computer may as well be programmed to calculate the surface
30  that each type of particles occupy. In the case of known
indices of refraction, the computer may also be programmed
to calculate the volume ratio between the two particles.

The method according to the invention lends itself to
automatization so that the control of the blend of par-
35  ticles of substances in a batch may be controlled con-

tinuously over time by arranging samples continuously and performing the method as described above. Up to now, there is no reliable real time method of making such measurements.

5       The invention has been described above with reference to a specific embodiment of the invention shown on the drawings. However, a skilled person reading this specification may realize other combinations of the different features indicated in the embodiment, and such other combina-
10      tions are intended to be within the scope of the invention. The invention is only limited by the appended patent claims.